Day Two: Lab Protocols

Supplies List

In order to do a laboratory day in the classroom, we rented a DNA Biotechnology Kit from the Fralin Institute. The kit included the following items along with an instruction booklet.

Description	Quantity
Microcentrifuge	1
Electrophoresis power supplies	4
Electrophoresis chambers	8
Mini-Pro Light Boxes	4-8
Thermal Grippers	2
Fisher Micropipettor (5-4 microliters)	1
Graduated cylinder, 100 ml	1
Graduated cylinder, 10 ml	1
10-microliter fixed volume pipettors	8
Yellow tips	1 bag
Microcentrifuge tube racks	8
Microcentrifuge tubes (1.5 ml)	1 pack
Colored microcentrifuge tubes (1.5 ml)	1 pack
Practice pipetting gels	1 pack
Non-mercury thermometer	1
Plastic boxes	8
Sharpie and label tape	1
DNA samples	100 microliters
1 kb Plus DNA ladder	100 microliters
Agarose	5 g
50x TAE buffer	25 ml
Practice pipetting dye	5 ml
50x methylene blue (reuseable)	8 ml

We also brought the following items from our lab.

- Agar Plates with Ampicillin
- pUC19 plasmids
- pUC19 plasmids with RFP construct
- Spreaders
- Extra TAE buffer
- Luria Broth
- Competent Cells (Transported with Dry Ice in Styrofoam)
- Micropipettes (varying sizes)
- Tips
- Erlenmeyer flasks

Protocols

Gel Preparation

We prepared 6-well agarose gels for the gel electrophoresis activity using the following protocol. Gels were prepared before and during the restriction enzyme digest activity by a team member.

1. Add 0.27 g of agarose to 30 ml 1x TAE buffer in Erlenmeyer flask.
2. Heat solution in microwave to dissolve agarose, swirling frequently.
3. Cool agarose slightly before pouring into gel molds.
4. Pour into gel mold and insert comb; gels will be ready in 15-20 minutes.
5. After cooling, molds were removed, and chambers were filled with 0.25x TAE.

Gel Electrophoresis

Students loaded 10 microliters of three different samples and 5 microliters of ladder into the wells using set volume micropipettes provided in the kit. Gels were run at 200 volts. Gels finished running in 20-25 minutes.

Gel Staining

Gels were placed in plastic boxes and covered with 1x methylene blue solution for 15-20 minutes by an instructor. Students then rinsed the gels with water repeatedly until bands were visible. Gels were then placed on light boxes for further visualization.

Transformation

Students completed the following:

1. Add 5uL of ligation product to 50uL of competent cells.
2. Gently flick the cells 6 times to properly mix the cells and the ligation products.
3. Incubate on ice for up to 5 minutes (NO LONGER). Then heat shock the samples at 42 C for 1 minute using one of the water baths. Place the samples back on ice for 1 minute, then place them in a tube rack (at room temperature). The bacterial cells do not tolerate the agarose well so keeping them on ice for too long can lead to disastrous results.
4. Add 1 mL of LB liquid to each sample.

Plating

Students completed the following:

1. Pipet 200uL of transformant onto a room temperature LB+antibiotic plate.
2. Use a glass pipet as a spreader and spread the pipetted transformants around the plate.